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14. **ABSTRACT** Recently we have shown that expression levels of both cannabinoid receptors CB and CB12 are higher in human prostate cancer cells than in normal prostate epithelial cells and treatment of LNCaP cells with WIN-55,212-2 (a mixed CB1/CB2 agonist) resulted in inhibition of cell growth and induction of apoptosis. Based on these data we suggested that WIN-55,212-2 or other non-habit forming cannabinoid receptor agonists could be developed as novel therapeutic agents for the treatment of prostate cancer (Sarfaraz et al., 2005). To understand the mechanistic basis of these effects here we show that WIN-55,212-2 (1-10 μM) treatment of LNCaP cells resulted in (i) upregulation p27/KIP1, (ii) down-regulation of cyclin E, D1, and D2, (iii) decrease in the protein expression of cyclin-dependent kinase 2,4, and 6, (iv) downregulation of pRb, (v) decrease in E2F (1-4) family of transcriptional factors and their heterodimeric partners DP-1 and DP-2, (vi) upregulation of Bax with concomitant downregulation of Bcl-2, favoring shift in Bax/Bcl-2 ratio more towards apoptosis, (vii) induction of apoptosis inducing factor, (viii) down regulation of caspases 3, 6, 7, and 9, and (ix) cleavage of poly (ADP- ribose) polymerase (PARP). Taken together, our data shows the involvement of two distinct pathways through which WIN-55,212-2 induces apoptosis. In the first pathway, G1 arrest and cell cycle dysregulation leads to the induction of apoptosis and in the second pathway apoptosis is mediated via activation of caspases. These data could lead to the development of novel mechanism based approaches for the treatment of prostate cancer.

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Background

Because prostate cancer has become the most common cancer diagnosed in men, developing novel targets and mechanism based agents for its treatment has become a challenging issue. In recent years cannabinoids, the active components of *Cannabis sativa linnaeus* (marijuana) and their derivatives are drawing renewed attention because of their diverse pharmacological activities such as cell growth inhibition, anti-inflammatory effects and tumor regression. Cannabinoids have shown to induce apoptosis in gliomas, PC-12 pheochromocytoma, CHP 100 neuroblastoma and hippocampal neurons in vitro, and most interestingly, regression of C6-cell gliomas in vivo. Further interest in cannabinoid research came from the discovery of specific cannabinoid system and the cloning of specific cannabinoid receptors (Guzman et al. 2001). These diversified effects of cannabinoids are now known to be mediated by the activation of specific G protein-coupled receptors that are normally bound by a family of endogenous ligands, the endocannabinoids (Porter and Felder 2001). Two different cannabinoid receptors have been characterized and cloned from mammalian tissues: the "central" CB_I receptor, and the "peripheral" CB_2 receptor.
Recently we have shown that expression levels of both cannabinoid receptors CB_1 and CB_2 are higher in human prostate cancer cells than in normal prostate epithelial cells and treatment of LNCaP cells with WIN-55,212-2 (a mixed CB_1/CB_2 agonist) resulted in inhibition of cell growth and induction of apoptosis. Based on these data we suggested that WIN-55,212-2 or other non-habit forming cannabinoid receptor agonists could be developed as novel therapeutic agents for the treatment of prostate cancer (Sarfaraz *et al.*, 2005). To understand the mechanistic basis of these effects here we show that WIN-55,212-2 (1-10 µM) treatment of LNCaP cells resulted in (i) upregulation p27/KIP1, (ii) down-regulation of cyclin E, D1, and D2, (iii) decrease in the protein expression of cyclin-dependent kinase 2, 4, and 6, (iv) downregulation of pRb, (v) decrease in E2F (1-4) family of transcriptional factors and their heterodimeric partners DP-1 and DP-2, (vi) upregulation of Bax with concomitant downregulation of Bcl-2, favoring shift in Bax/Bcl-2 ratio more towards apoptosis, (vii) induction of apoptosis inducing factor, (viii) down regulation of caspases 3, 6, 7, and 9, and (ix) cleavage of poly (ADP- ribose) polymerase (PARP). Taken together, our data shows the involvement of two distinct pathways through which WIN-55,212-2 induces apoptosis. In the first pathway, G_1 arrest and cell cycle dysregulation leads to the induction of apoptosis and in the second pathway apoptosis is mediated via activation of caspases. These data could lead to the development of novel mechanism based approaches for the treatment of prostate cancer.

The major purpose of this research supported by the Award W81XWH-04-1-0217 is to establish whether cannabinoid receptors could prove to be useful targets for the treatment of prostate cancer.

Body

Specific Aims: The following specific aims were proposed

1. To investigate the consequences of the activation of cannabinoid receptors in human prostate cancer cells *in vitro*.

(a) To investigate whether the activation of cannabinoid receptors impart inhibitory effect on cell growth/cell viability in human prostate cancer cells without affecting normal cells.

(b) To investigate whether cannabinoids selectively induces apoptosis in human prostate carcinoma cells without affecting normal cells.

(c) To investigate whether cannabinoids is associated with inhibition of angiogenesis and PSA levels in human prostate carcinoma cells.

2. To investigate the consequences of the activation of cannabinoid receptors under *in vivo* situation, in athymic nude mice implanted with human prostate cancer cells.

Animal work in progress.

Materials and Methods

Materials

R-(+)-WIN 55,212-2 (2, 3 Dihydro-5-methyl -3 ([morpholinyl]methyl) pyrollo (1,2,3 de)-1,4-benzoxazinyl]- [1-napthaleny] methanone, $C_{27}H_{26}N_2O_3.CH_3SO_3H$ was purchased from Sigma Chemical Company (St. Louis, MO). Dulbecco's Modified Eagle's Medium and Fetal Bovine Serum (FBS) were procured from Invitrogen Corporation (Grand Island, N.Y). Antibiotic (Penicillin and Streptomycin) used were obtained from Cellgro Mediatech, Inc. (Herndon, VA). The mono- and polyclonal antibodies (cdk2, 4 and 6, KIP1/p27, E2F-3 and DP-2) were obtained from Santa Cruz Biotechnology Inc. CA. The human reactive monoclonal and polyclonal antibodies (cyclins D1, D2, E, pRb, E2F-1, E2F-2, E2F-4 and DP-1) were obtained from Labvision (Fremont, CA). Monoclonal and polyclonal antibodies for anti-PARP, Bcl-2 Bax were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-mouse secondary horseradish peroxidase conjugate was obtained from Amersham Biosciences Limited (Buckinghamshire, England). Protein was estimated using BCA Protein assay kit obtained from Pierece (Rockford, IL).

Cell culture

LNCaP cells obtained from ATCC (Manassas, VA) were cultured in Dulbecco's Modified Eagle's Medium supplemented with 5% heat-inactivated fetal bovine serum and 1% antibiotic penicillin and streptomycin. The cells were maintained under standard cell culture conditions at 37 ^{0}C and 5% CO_2 in a humid environment.

Treatment of cells

WIN-55,212-2, (dissolved in DMSO) was used for the treatment of cells. The final concentration of DMSO used was 0.1% (v/v) for each treatment. For dose-dependent studies cells were treated with WIN-55,212-2 at 1.0, 2.5, 5.0, 7.5, 10.0 μM final concentrations for 24 h in complete cell medium. Control cells were treated with vehicle alone.

Cell viability

The cells were grown at density of 1 X 10^6 cells in 100 mm culture dishes and treated with WIN-55,212-2 (1-10 μM) for 24 h. The cells were trypsinized and collected in the microfuge tube. The cells were pelleted by centrifugation, and the cell pellet was resuspended in phosphate-buffered saline (PBS) (300 μl). Trypan blue (0.4% in PBS; 10 μl) was added to a smaller aliquot (10 μl) of cell suspension, and the number of cells (viable-unstained and nonviable-blue) were counted using a haemocytometer.

Cell cycle analysis by flow cytometry

The cells were grown at density of 1 X 10^6 cells in 100 mm culture dishes and were treated with WIN-55,212-2 (1.0, 2.5, 5.0, 7.5, 10.0 μM doses) for 24 h. The cells were trypsinized, washed with PBS, and processed for labeling with fluorescein-tagged deoxyuridine triphosphate nucleotide and propidium iodide by use of an Apo-direct apoptosis kit obtained from Phoenix Flow Systems (San Diego, CA) as per manufacturer's protocol. The labeled cells were analyzed using a FACScan benchtop cytometer (BD Biosciences, San Jose, CA) at the UWCCC Flow Cytometry Facility in the University of Wisconsin. The analyses were performed using ModFit LT software (Verity Software House, Topsham, ME) for cell cycle analysis.

Detection of cleaved caspase-3 by confocal microscopy

The cells were grown in two chambered cell culture slides (BD Biosciences, Bedford, MA) and were treated with WIN-55,212-2 (5.0, 7.5, 10.0 μM doses) for 24 h, washed with 1x PBS at room temperature and were immediately fixed with cold 100% methanol at -20 ^{0}C for 10 minutes. Cells were blocked with blocking buffer (5.5% normal goat serum in TBST) for 45-60 min and were washed with TBS. Cells were then incubated with primary antibody Alexa Fluor 488 conjugate, (Cell Signaling Technology, Beverly, MA) overnight using vendor's protocol. After incubation cell were washed twice for 5 minutes with TBST and once with TBS. Coverslips were mounted using the Prolong Antifade kit obtained from Molecular Probes, (Eugene, OR). Cells were visualized with a Bio-Rad MRC1000 scan head mounted transversely to an inverted Nikon Diaphot 200 microscope at the Keck Neural Imaging Lab in the University of Wisconsin.

Preparation of cell lysates and western blot analysis

Following treatment of cells with WIN-55,212-2, the medium was aspirated and the cells were washed with cold PBS (10 mmol/l, pH 7.45). The cells were then incubated in ice cold lysis buffer (50 mmol/l Tris-HCl, 150 mmol/l NaCl, 1 mmol/l EGTA, 1 mmol/l EDTA, 20 mmol/l NaF, 100 mmol/l Na_3VO_4, 0.5% NP-40, 1% Triton X-100, 1mmol/l phenyl methyl sulfonyl fluoride (PMSF) (pH 7.4), with freshly added protease inhibitor cocktail (Protease Inhibitor Cocktail Set III, Calbiochem, La Jolla, CA) over ice for 20 minutes. The cells were scraped and the lysate was collected in a microfuge tube and passed through a 21.5 G needle to break up the cell aggregates. The lysate was cleared by centrifugation at 14000 x g for 15 minutes at 4 ^{0}C, and the supernatant (total cell lysate) collected, aliquoted and was used on the day of preparation or immediately stored at -80 ^{0}C for use at a later time. For western blotting, 25-50 μg protein was resolved over 12 % polyacrylamide gels and transferred onto a nitrocellulose membrane. The non-specific sites on blots were blocked by incubating in blocking buffer (5% non fat dry milk/1% Tween 20 in 20 mmol/l TBS, pH 7.6) for 1 hour at room temperature, incubated with appropriate monoclonal primary antibody in blocking buffer for 90 minutes to overnight at 4 ^{0}C, followed by incubation with anti-mouse or anti-rabbit secondary antibody horse-radish peroxidase conjugate and detected by chemiluminescence and autoradiography using Hyperfilm obtained from Amersham Biosciences (UK Ltd.). Densitometric measurements of the bands in western blot analysis were performed using digitalized scientific software program UN-SCAN-IT purchased from Silk Scientific Corporation (Orem, UT).

Results

(1) WIN-55,212-2 causes cell growth inhibition and G_1 phase arrest in LNCaP cells

To evaluate the cell viability response of WIN-55,212-2 on LNCaP cells, trypan blue exclusion assay was employed. Data in Fig 1A shows that treatment of LNCaP cells with

WIN-55,212-2 (1–10 μM) for 24 h significantly decreased the viability. Several studies have shown that the induction of apoptosis may be cell cycle dependent (Hartwell and Kastan, 1994; Morgan and Kastan, 1997; King and Cidlowski, 1998; Sandhu and Slingerland, 2000; Vermeulen *et al.,* 2003). Therefore, in next series of experiments, we tested the hypothesis that WIN-55,212-2, caused apoptosis of LNCaP cells is mediated via cell cycle blockade. We therefore performed DNA cell cycle analysis to assess the effect of WIN-55,212-2 treatment on the distribution of cells in the cell cycle. As shown in Fig 1B, compared with vehicle treatment, WIN-55,212-2 treatment was found to result in dose-dependent accumulation of cells in G_1 phase of the cell cycle (59%, 62%, 69%, 81% and 83% cells at 1.0, 2.5, 5.0, 7.5 and 10 μM concentrations, respectively, Fig 1B) and increase in apoptosis at 7.5 and 10 μM doses. This observation is important because the molecular analyses of human cancers have revealed that cell cycle regulators are frequently mutated in most common malignancies (Kastan *et al.,* 1995; Molinari M, 2000). Therefore, in recent years, inhibition of the cell cycle has been appreciated as a target for the management of cancer (McDonald *et al.,* 2000; Owa *et al.,* 2000).

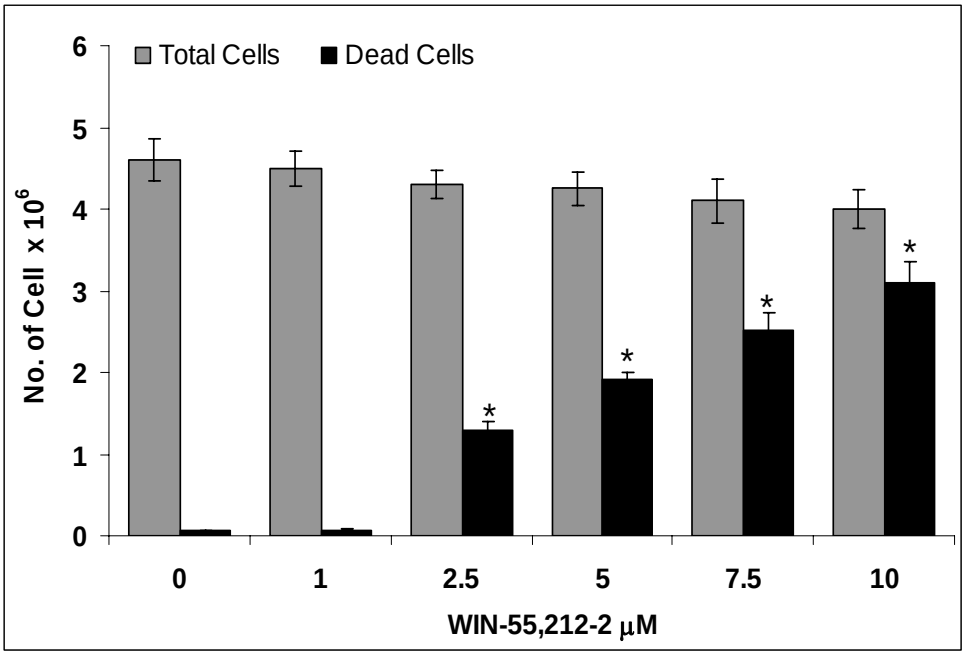


*Fig 1A Effect of WIN-55,212-2 treatment on cell growth in LNCaP cells. Cell growth inhibition was ascertained by trypan blue exclusion assay. The cell growth inhibition data shown are mean ±S.E. of three independent experiments. * indicates p < 0.001 compared with control.*

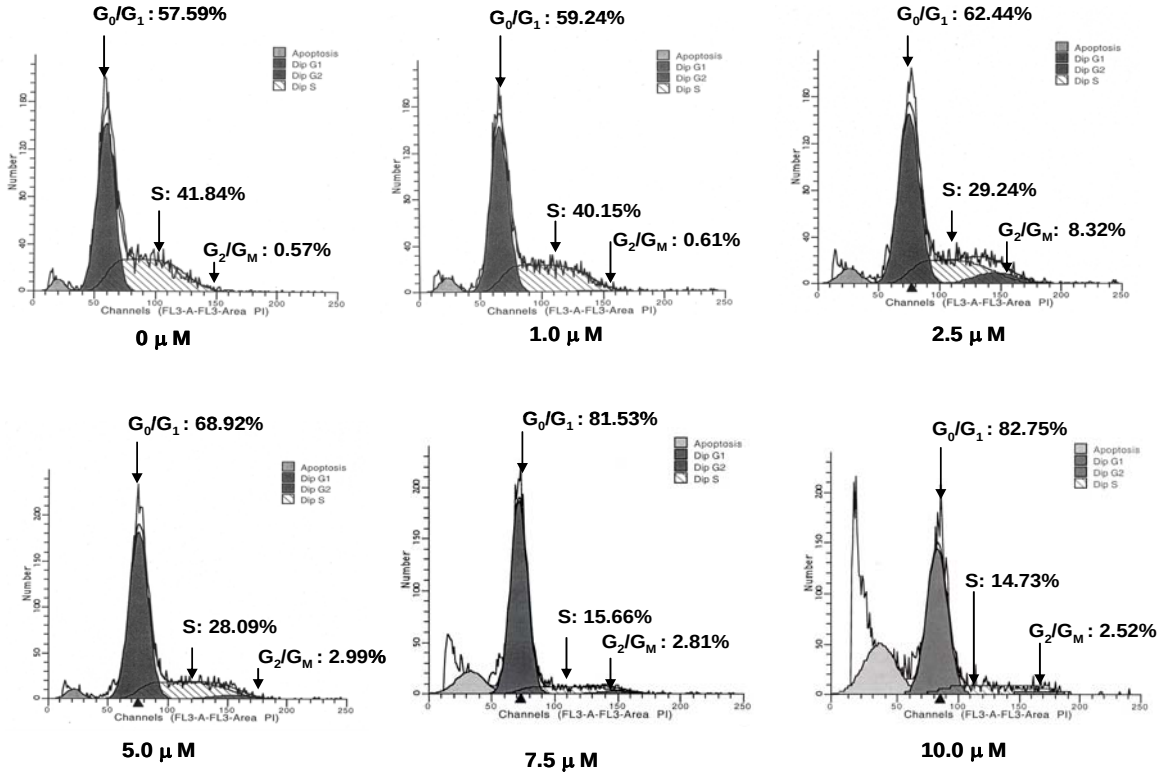


*Fig 1B Effect of WIN-55,212-2 treatment on (A) cell growth and (B) cell cycle in LNCaP cells. Cell growth inhibition was ascertained by trypan blue exclusion assay. The cell growth inhibition data shown are mean ±S.E. of three independent experiments. * indicates p < 0.001 compared with control. Cell cycle analysis was performed by flow cytometry as detailed in Materials and Methods. The labeled cells were analyzed using a FACScan benchtop cytometer and percentage of cells in G_0–G_1, S and G_2-M phase were calculated using ModFit LT software. The data shown here are from a typical experiment repeated three times.*

(2) WIN-55,212-2 induced cell cycle arrest is mediated via an induction of KIP1/p27 and concomitant inhibition in cyclins D1, D2, E and cdk2, cdk4 and cdk6

Because our studies demonstrated that WIN-55,212-2 treatment of cells resulted in a G_1-phase cell cycle arrest and apoptosis, we examined the effect of WIN-55,212-2 on cell cycle-regulatory molecules operative in G_1 phase of the cell cycle. Studies have shown the critical role of p27/KIP1 in apoptosis and cell cycle progression through G_0-G_1 phase (Macri and Loda, 1998; Pavletich NP, 1999; Atallah *et al.*, 2004). We observed a significant induction of p27/KIP1 by WIN-55,212-2 at 7.5 and 10 μM doses. (Fig 2A). Relative density data revealed an increase of 2.3 and 2.6 folds in the protein expression of Kip/p27 at 7.5 μM and 10 μM concentrations, respectively. Using immunoblot analysis, we also assessed the effect of WIN-55,212-2 treatment on the protein expression of the cyclins and cdks, which are known to be regulated by KIP1/p27. WIN-55,212-2

treatment of the cells resulted in a dose-dependent decrease in protein expressions of cyclin D1, cyclin D2, and cyclin E (Fig. 2B) as well as cdk2, cdk4, and cdk6 (Fig. 2C) . Densitometric analysis data of cyclins revealed a significant decrease in the expression of cyclin D1 (84%, 97%), cyclin D2 (60%, 86%) and cyclin E (40%, 50%) at 7.5 and 10.0 μM concentrations of WIN-55,212-2, respectively (Fig 2B). Relative density data of cdks also revealed a significant decrease in the expression of cdk2 (43%, 65%), cdk4 (54%, 89%) and cdk6 (46%, 60%) at similar doses of WIN-55,212-2.

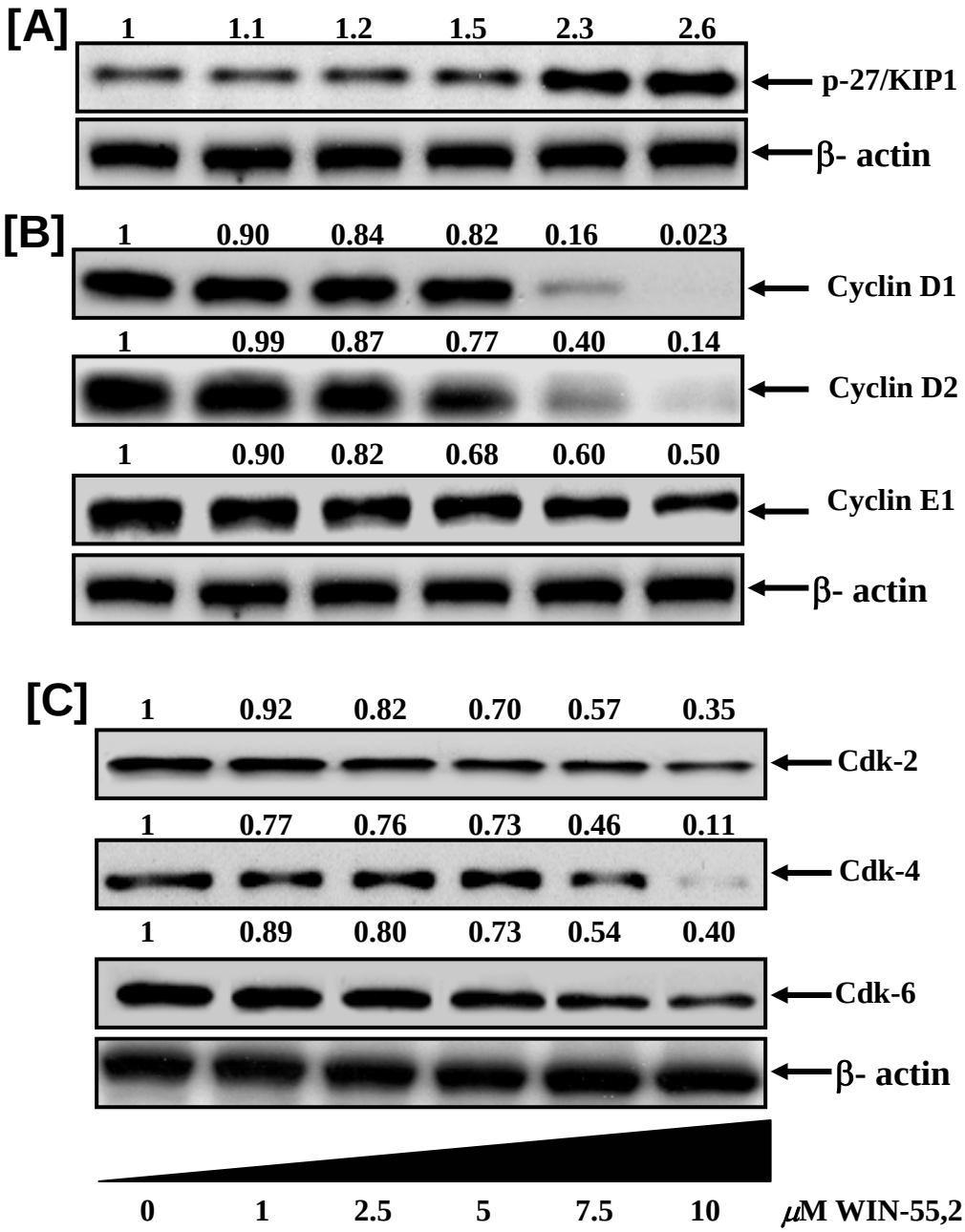
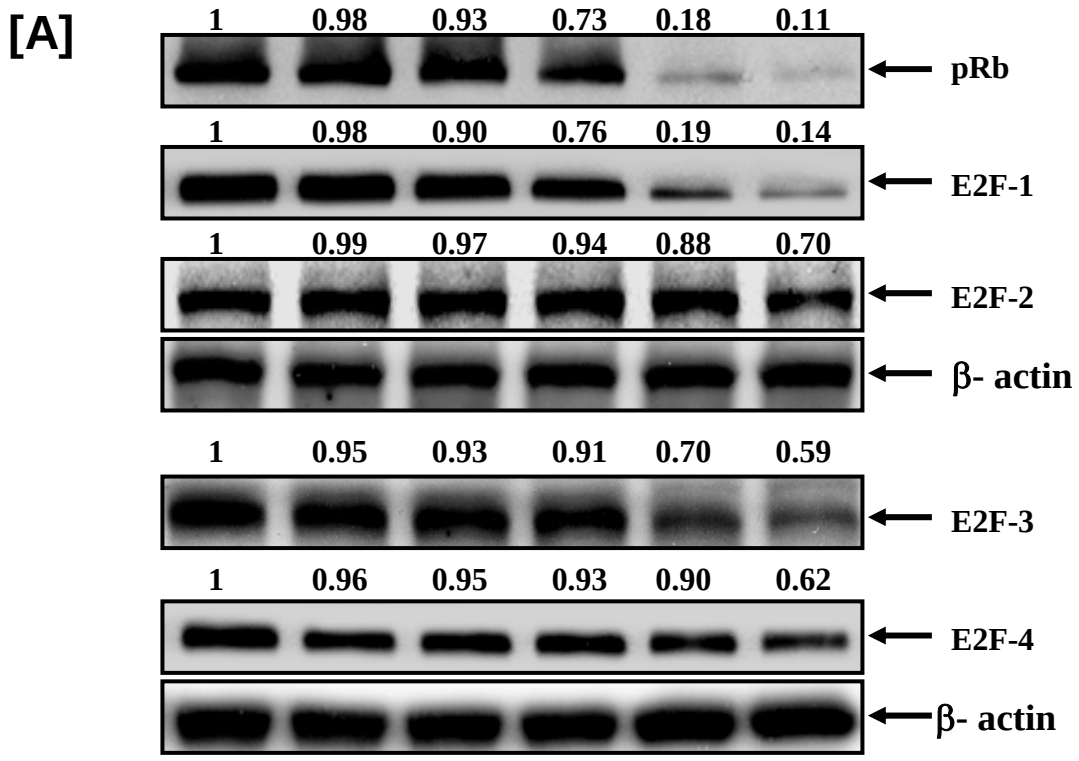


Fig 2 Effect of WIN-55,212-2 treatment on protein expression of (A) KIP1/p27, (B) cyclinD1, D2 and E and (C), cdk 2, 4 & 6 in LNCaP cells. As detailed in "Materials and Methods" the cells were treated with DMSO alone or specified

concentrations of WIN-55,212-2 and total cell lysates were prepared for immunoblot analysis. The values above the figures represent relative density of the bands normalized to β-actin. The data shown here are from a representative experiment repeated three times with similar results.

(3) WIN-55,212-2 inhibits protein expression of pRB, E2F and DP

Downregulation of cdk4/6 has been shown to be associated with a decrease in the expression of a key regulator of the $G_1 \rightarrow S$ phase transition in the cell cycle, the retinoblastoma (pRb) tumor suppressor protein (Nevins *et al.,* 1997; Deshpande *et al.,* 2005). Therefore, we next examined the effect of WIN-55,212-2 on pRb. Immunoblot analysis data revealed that WIN-55,212-2 treatment of LNCaP cells resulted in a significant decrease in the protein expression of pRb. Densitometric analysis of immunoblots showed 82% and 89% inhibition at 7.5 and 10 μM concentrations of WIN-55,212-2 (Fig 3A). Since pRb controls cell cycle by binding to and inhibiting the E2F transcription factors, we evaluated the status of the protein expression of E2F (1-4) transcription factors. As shown in Fig 3A, WIN-55,212-2 treatment of cells resulted in a dose-dependent decrease in E2F transcription factors. Relative density data revealed an inhibition in E2F-1 (81%and 86%), E2F-2 (12% and 30%) E2F-3 (30% and 41%) and E2F-4 (10% and 38%) at a concentration of 7.5 μM and 10 μM of WIN-55,212-2. Since the activity of E2F is known to be dependent on its heterodimeric association with members of DP family of proteins, we also evaluated the effect of WIN-55,212-2 treatment on both members of DP family viz. DP-1 and DP-2. Immunoblot and densitometric analysis data revealed a decrease in the protein expression of DP-1 (37% and 48%) and DP-2 (30% and 56%) at 7.5 μM and 10 μM concentration of WIN-55,212-2 (3B).



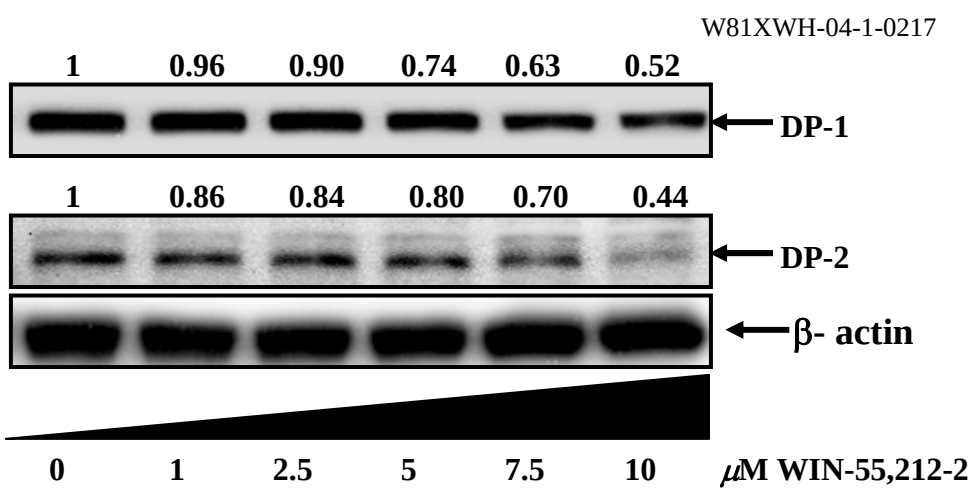


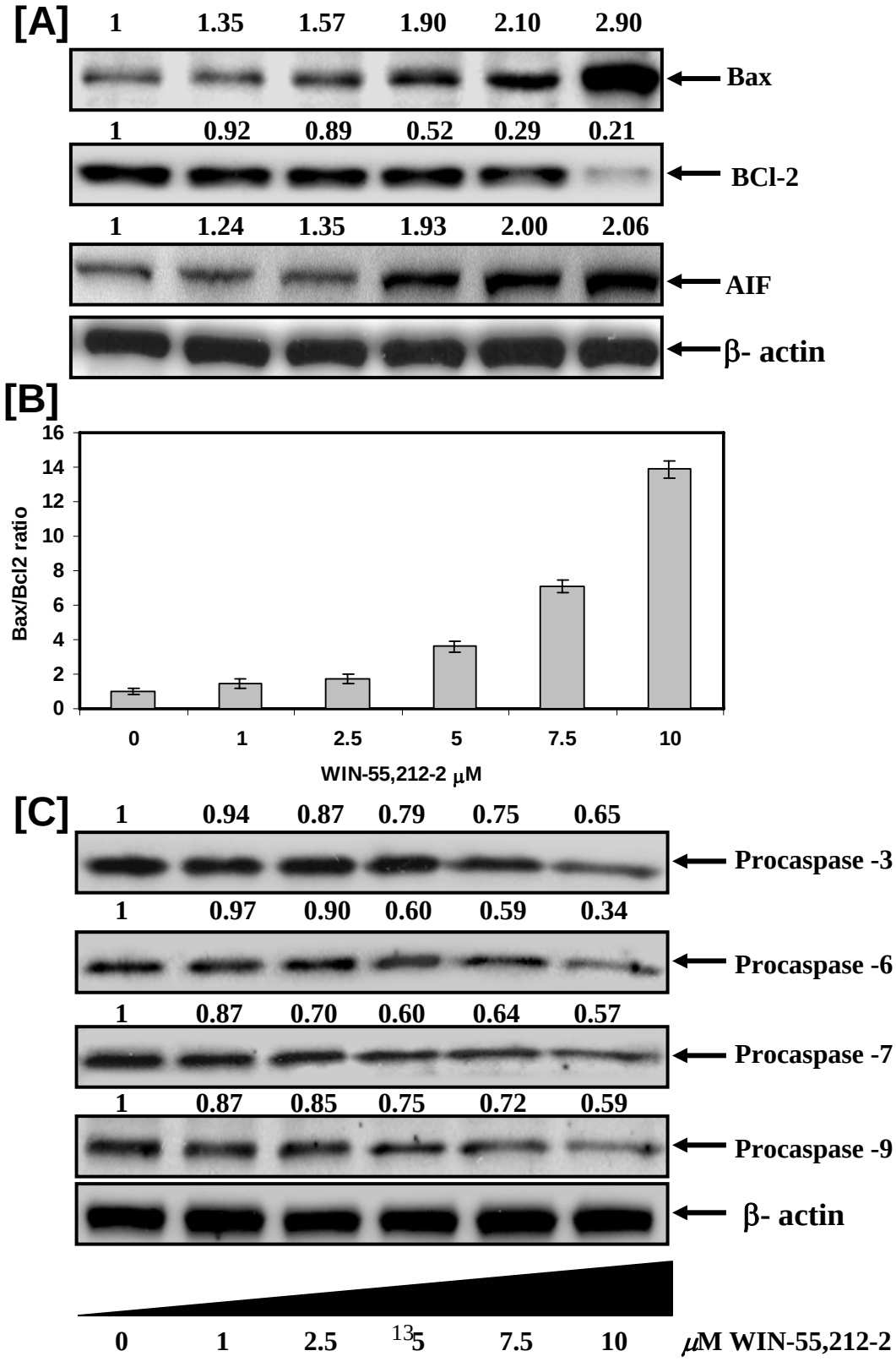
Fig 3 Effect of WIN-55,212-2 treatment on protein expression of (A) pRb and E2F (1-4) (B) DP1 and DP2, in LNCaP cells. As detailed in "Materials and Methods" the cells were treated with DMSO alone or specified concentrations of WIN-55,212-2 and total cell lysates were prepared for immunoblot analysis. The values above the figures represent relative density of the bands normalized to β-actin. The data shown here are from a representative experiment repeated three times with similar results.

(4) WIN-55,212-2 induces apoptosis via classical apoptotic pathway

The above data suggest that WIN-55,212-2 induces growth inhibition via cell cycle arrest in G_1 phase of the cell cycle followed by apoptosis. Since Bax and Bcl-2 play a crucial role in apoptosis, we next determined the effect of WIN-55,212-2 treatment of LNCaP cells on protein levels of Bax and Bcl-2. The Western blot analysis exhibited a significant increase in the protein expression of Bax at 7.5 and 10 μM concentration of WIN-55,212-2 (Fig 4A). In sharp contrast, the protein expression of Bcl-2 was significantly decreased by WIN-55,212-2 treatment in a dose-dependent fashion (Fig 4A). A significant dose-dependent shift in the ratio of Bax to Bcl-2 was observed after WIN-55,212-2 treatment indicating the induction of apoptotic process (Fig 4B). Relative density data revealed an increase in protein expression of Bax by 2.1 and 2.9 folds with concomitant decrease in Bcl-2 protein expression by 71% and 79% at a dose of 7.5 and 10 μM respectively. Decrease in Bcl-2 expression was associated with increase in AIF to 2.0 and 2.06 folds at the above mentioned doses of WIN-55,212-2 (Fig 4A).

Alteration in Bax/Bcl-2 is known to initiate caspase signaling, therefore, we evaluated the involvement of various caspases during WIN-55,212-2-mediated apoptotic death of LNCaP cells. As shown by the immunoblot analysis, WIN-55,212-2 treatment was found to result in a significant decrease in the pro form of caspase-3, caspase-6, caspase-7 and caspase-9 at a concentration of 7.5 and 10μM (Fig 4C). To assess possible involvement of caspase-3 activation in apoptosis, we next measured cleaved caspase-3 immunostaining. Cells were stained with Alexa Fluor 488 conjugate antibody and were viewed under confocal microscope. Intensity of the active caspase-3 staining was higher in cells treated with 7.5 and 10μM concentration of WIN-55,212-2 as compared to that at lower concentrations of WIN-55,212-2 and control (Fig 4D). The downstream signals

during apoptosis are transmitted via caspases, which upon conversion from pro to active forms mediate the cleavage of PARP. We found that WIN-55,212-2 treatment caused cleavage of 116 KD PARP to 85 KD (Fig 4E). Relative density data revealed a decrease in the protein expression of PARP (116KD) (49% and 81%) with a concomitant increase in its cleaved product (85KD) by 3.1 and 4.4 folds at a concentrations of 7.5 and 10 μM, respectively.



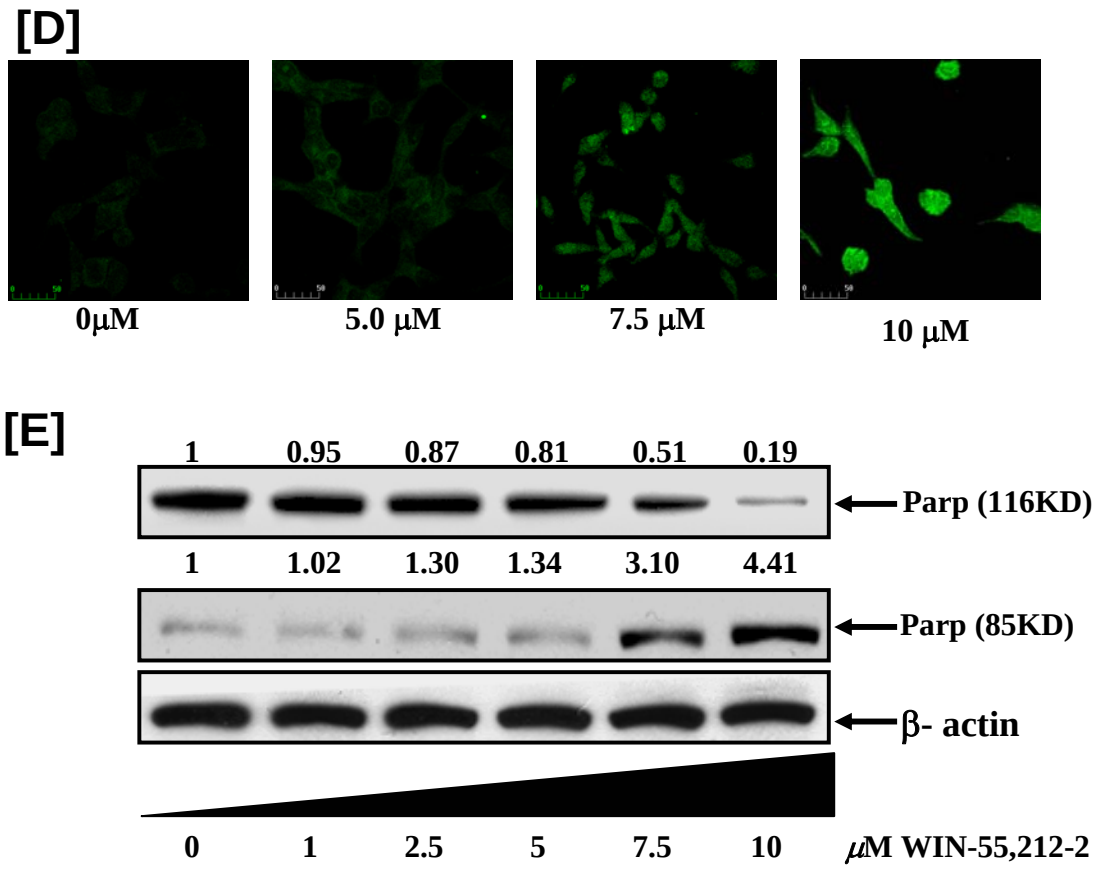


Fig 4 Effect of WIN-55,212-2 treatment on (A) protein expression of Bax, Bcl-2 and AIF (B) Bax/BCl-2 ratio (C) protein expression of caspase-3, 6, 7, 9 (D) cleaved caspase-3 and (E) cleavage of PARP. As detailed in "Materials and Methods" the cells were treated with DMSO alone or specified concentrations of WIN-55,212-2 and total cell lysates were prepared for immunoblot analysis. The values above the figures represent relative density of the bands normalized to β-actin. The data shown here are from a representative experiment repeated three times with similar results. The data obtained from the immunoblot analyses of Bax and Bcl-2 were used to evaluate the effect of WIN-55,212-2 on the Bax/Bcl-2 ratio. The densitometric analysis of Bax and Bcl-2 bands was performed using UN-SCAN-IT software (Silk Scientific, Inc., Orem, UT), and the data (relative density normalized to β-actin) were plotted as Bax/Bcl-2 ratio. Detection of cleaved caspase-3 by confocal fluorescence microscopy; cells were treated with WIN-55,212-2 5.0, 7.5 and 10 μM for 24 h and were stained with antibody Alexa Fluor 488 conjugate.

Key Research Accomplishments

(i) This study was conducted to understand the mechanistic basis of cannabinoid receptor-agonist induced prostate cancer cell growth inhibition and induction of apoptosis.

(ii) Treatment of cells with WIN-55,212-2 was found to result in (a) an arrest of the cells in the G_0/G_1 phase of the cell cycle; (b) up regulation of ERK1/2, JNK1/2, p38; and (c) inhibition of PI3k/Akt pathways.

(iii) To define the involvement of regulatory proteins operative in the G_0/G_1 phase of the cell cycle, we next determined the effects of WIN-55,212-2 treatment of cells on cyclin kinase inhibitor (cki)-cyclin-cyclin dependent kinase (cdk) machinery. We observed that WIN-55,212-2 (1-10 μM) treatment resulted in (a) an induction of p27/KIP1; (b) down-regulation of cyclin D1, D2, E; and (c) decrease in the expression of cdk -2, -4, and -6.

(iv) Western blot analysis showed a decrease in the protein expression of (a) pRb; (b) E2F (1 through 4); and (c) DP1 and DP2.

(v) WIN-55,212-2 treatment of cells resulted in a dose-dependent increase in Bax/Bcl-2 ratio in such a way that favors apoptosis. The induction of apoptosis proceeded through down regulation of caspases 3, 6, 7, and 9 and cleavage of PARP.

(vi) Taken together, our data suggests the involvement of two distinct pathways through which WIN-55,212-2 induces apoptosis of LNCaP cells. In the first pathway, activation of ERK1/2 leads to cell cycle dysregulation and arrest and in the second pathway up regulation of Bax/Bcl2 ratio and activation of caspases results in an induction of apoptosis.

Reportable Outcomes

Work described in this report is ready for communication to a peer-reviewed journal for its consideration for publication. In addition an abstract has been submitted for poster presentation at the Annual meeting of "American Association of Cancer Research" to be held in Washington D.C from 1st to 5th April 2006.

Conclusions

Based on the outcome of this study and the available literature knowledge, and as shown in the composite scheme in Fig 5, we suggest that two distinct pathways are operational through which cannabinoid receptor agonist WIN-55,212-2 results in apoptotic cell death in LNCaP cells. One likely mechanism is the induction of cyclin kinase inhibitor p27, which inhibits cell cycle regulatory molecules resulting in G_1 arrest and apoptosis. Downregulation of cdk4/6 inhibits of pRb which downregulates E2F family of proteins and its heterodimeric partners DP1 and DP2 leading to gene transcription and apoptosis. The second likely mechanism appears to be modulated by Bax and Bcl-2 proteins which activates caspases resulting in apoptotic cell death. Hence, we conclude that cannabinoids

should be considered as agents for the management of prostate cancer. If our hypothesis is supported by *in vivo* experiments then long term implications of our work could be to develop non-habit forming cannabinoid agonist (s) for the management of prostate cancer.

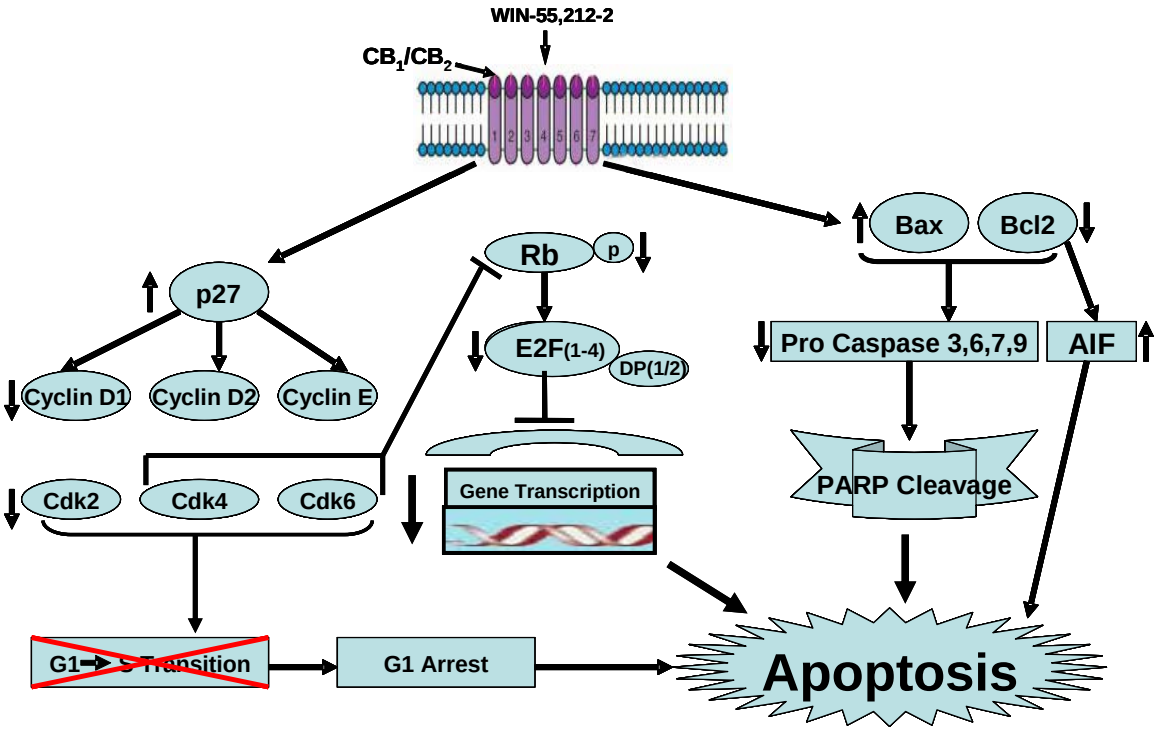


Fig 5 Proposed schematic model for WIN-55,212-2-mediated cell cycle dysregulation and induction of apoptosis.

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